EXPLORATION OF CARDIAC BIOMARKERS TO DECLINE MORTALITY IN COVID 19 PATIENTS

Sonila Khan[1], Rabia Yaqoob[2], Zelle Humma[3]

[1]Student of MPhil Biochemistry, Department of Biochemistry and Biotechnology, Women University Multan, Pakistan
[2] Student of MPhil Biochemistry, Department of Biochemistry and Biotechnology, Women University Multan, Pakistan
[3] Assistant Professor at Department of Biochemistry and Biotechnology, Women University Multan, Pakistan

1sonilakhan789@gmail.com
[2]tayyabchandia9994@gmail.com
[3]hummabiochem@gmail.com

Abstract— COVID 19 disease is caused by SARS CoV-2 had been started from Wuhan, a city of China and affected hundreds of countries has no therapeutic agents known for the treatment. [2] The mortality rate of disease is high with the patients with pre-existing cardiovascular diseases. In some cases, COVID 19 is associated with induction of acute COVID 19 cardiovascular syndromes. The mortality risks of disease can be lessened by early detection of cardiovascular diseases and myocardial injuries by elevated level of cardiac biomarkers more specifically cardiac troponin I (cTnI) and B type natriuretic peptides (BNP)[4] and N terminal pro BNP NT pro BNP) [7]. Virus enters the cardiac cells by specific binding with spike proteins and caused increase destruction of myocardial cells and leading toward the release of specific cardiac troponin in the blood stream.

Keywords—A disintegrin and metalloprotease 17, angiotensin converting enzyme 2, brain natriuretic peptides, cardiotropins, cardiovascular disease

I. INTRODUCTION

COVID 19 is the widespread disease that had affected the world, initiated from the Wuhan city of China in December 2019. [12] Corona virus disease 2019 (COVID) is caused by the SARS CoV-2 stands for severe acute respiratory syndrome coronavirus 2, spread of the disease had affected the world. [3,1,7] Similar type of disease originated in 2003-2004 in China and then Saudi Arabia was MERS-CoV. [1] The patients affected by SARS CoV-2 with appearance of symptoms including fever, fatigue, cough, and pneumonia, organ disfunction in the first weak after infection.[4] In order to prevent the fatality of the disease COVID 19 should be detected at initial stages by using specific methods using reverse transcription polymerase chain reaction (RT-PCR). [6,9]

Due to the severity of the disease various retrospective studies have been done on the COVID 19 patients indicated that along with the lung injury induced by COVID 19 level of cardiac biomarkers elevated. [1] This elevated level of cardiac biomarker is associated with high risk of mortality in COVID 19 cardiac patients. The condition become more severe due to poor prognosis of cardiovascular disease in COVID patients. SARS-CoV-19 infect the heart cell by different mechanisms result in permanent cardiomyocyte loss.[8]

II. CARDIAC BIOMARKERS

Cardiac biomarkers are said to be the specific biological analytes and elevated concentration of these biomarkers is detected in the blood stream of patient with cardiovascular disease. [2,4] Various biomarkers have been established in order to risk prediction tools in the diagnosis, treatment and research purposes. Glutamate oxaloacetic transaminase activity in serum is the earliest biomarker used since 1954. [10]

These biomarkers include:

1. Cardiac troponin (cTn)
2. Suppression of tumorigenicity 2 (ST2) [1]
3. Brain natriuretic peptide (BNP)
4. NT pro BNP [3]
5. Myeloperoxidase (MPO) cytokines
6. Plasminogen activator-1 (PA-1) [8,12]
7. Galectin 3 (G3) [8]
8. Creatinine kinase MB and myoglobin
9. Ischemia modified albumin [3]
10. Arterial natriuretic peptides (ANP)
11. High sensitivity C reactive protein (hs-CRP) [9]
12. Growth differentiation factor-15 (GDF-15)

III. CLASSIFICATION OF CARDIAC BIOMARKERS

A. Biomarkers of plaque instability/disruption
B. Biomarkers of inflammation
C. Biomarkers of myocardial ischemia
D. Hemodynamic markers [15,8,12]

A.Biomarkers of plaque instability/disruption

Elevated level of C-reactive protein [10], myeloperoxidase and soluble fragment CD40 ligand, are novel biomarker for inflammatory processes.[15]in response to interleukin 6, a pentameric C-reactive protein (CRP) produce in liver. MPO is the enzyme that is produce as reactive oxidized molecule in various inflammatory cells in response to the tissue neutrophils and monocytes. sCD40 is a signaling protein associated with inflammatory pathways of the cell. [1,12]

B. Biomarkers of plaque Instability/ disruption

In pregnant women, pregnancy-associated plasma protein A (PAPP-A) [17], are present and have the zinc binding domain, highly associated with the patients have unstable plaque. [14,3] Coronary plaque destabilization cascade mechanism includes the release of choline in blood stream due to action of phospholipase D enzyme is the good predictor of cardiovascular disease like myocardial infarction. [5,8]

C. Biomarkers of Myocardial Ischemia

Ischemia-modified albumin (IMA), is albumin in the serum of cardiovascular disease patient with low binding ability for the metal cobalt at the N terminal. This transition in metal binding ability of albumin protein is associated with the myocardial ischemia. [2,3, 14]

Serum screening of CVD person with myocardial infarction is done to determine the level of free fatty acids unbound to albumin (FFAu), [18] using ratio of serum albumin and free fatty acid ratio. The elevated level off FFAu is the biomarker for heart narcosis too. [6]

Heart-type fatty acid binding protein (H-FABP), a protein with small molecular weight and high stability is present in the cytoplasm of the heart cells of disease person. [6,4] Elevated level of H-FABP is associated with the increased cardiac events associated with the death of person. [9]

Creatine kinase MB and myoglobin, is least specific biomarker for detection of myocardial infarction and ischemia because of association of myoglobin with muscular injury and also due to the small size and high diffusion rate of myoglobin. [14,6,9] Creatine kinase muscle brain activity (CK-MB) and myoglobin is a good biomarker when linked with the other specific biomarker such as cardiac troponin. [11]

D. Hemodynamic Biomarkers

Natriuretic peptides in ischemia, associated with ventricular impairment, systolic dysfunction, ventricular relaxation impairment. These natriuretic peptides are formed by the ventricular stress and get elevated indicating above pathophysiological conditions. [16] In CVD patients the risk of high mortality is related to the level of cardiac biomarkers such as natriuretic peptides that includes B type natriuretic peptide (BNP) and N terminal NT pro B type natriuretic peptides (NT pro BNP) [8,12,16]

IV. BIOMARKERS OF GOLDSTANDARD

Cardiac troponin is the most specific cardiac biomarker used in the diagnosis and risk factor estimation of cardiovascular disease person. These biomarkers are of two types cTnT and cTnI and both are very specific even in low o very slight increase in level of creatine kinase MB they effectively diagnose the cardiovascular diseases and associated risk. [13] These biomarkers are also specific in diagnosis, prognosis and re-evaluation of the cardiovascular disease. Cardiac troponin also provides the indication of cell death in case of disease. [6,1] Along with BNP and NT pro BNP provide information about hemodynamic stress provided by the tissue damage and cell death. [2]

V. CARDIAC BIOMARKERS FOR COVID 19

In COVID 19 patient serum many of the cardiac biomarkers are seen elevated such as creatine kinase (CK), cardiac troponin-I (cTnI), creatine kinase muscle/brain activity (CK-MB), myoglobin (Mb), brain natriuretic peptides (BNP and NT pro BNP), aspartate amino transferase (AST), alpha hydroxy butyrate dehydrogenase (α-HBDH) and lactose dehydrogenase, had been seen in the COVID 19 patients. [9,16,12] Out of these estimated biomarkers in laboratory CK-MB, cTnI, BNP and NT pro BNP are specific biomarkers that seen elevated in the serum of COVID 19 patient. [11] Other biomarkers are not very specific to myocardial injury and also indicate the risk of diseases other than cardiovascular diseases. [14]

It has been estimated that the high level of these prognostic biomarkers predicts fatality rate in COVID -19 Patients. The increase in level of these specific biomarkers indicate the higher mortality risk in patients with cardiovascular disease. [16] Echocardiographic data is also an indicator that advanced level of cardiac biomarkers in the patient with acute SARS-CoV-2 infection is compatible with the acute cardiomyopathies. Continuous elevation of cardiac biomarkers in an indication to the acute cardiomyopathies in COVID 19 patients. [1, 2, 4] This data concludes that the patients with cardiovascular diseases with SARS-CoV-2 infection are more likely to develop high mortality risk ICU

cases. Continuous elevation in the level of cardiac biomarkers in COVID patients leads towards 13 percent higher mortality factor in ICU patients. [10]

VI. ADVAMTAGES OF BIOMARKERS

Cardiac biomarkers are biological parameters and their value in the serum indicate the high level of risk factors due to the myocardial injuries. Biomarkers are the screening, monitoring and diagnosis tool for the disease condition, pathogenic process and impairment of normal body functions. [2,8,9,12] Cardiac biomarkers are valuable tools used in the diagnosis, treatment and risk stratification of ICU patients, emergency conditions and myocardial injuries. These cardiac biomarkers are specific in providing new treatment guidelines in future perspective. [3,4,7] Severity of the disease is measured by cardiac biomarkers associated with the deaths in case of COVID 19. [1]

A. Association between COVID 19 and Cardiovascular disease

There is close association with disease severity and with already existing CVD in ICU patients in comparison to non-ICU patients and risk of acute COVID 19 cardiovascular syndrome increase. [1,4] In recent studies an association between SARS CoV 2 and acute cardiovascular diseases have been shown. SARS CoV 2 can induce acute COVID 19 cardiovascular syndromes by some unknown mechanisms, but the possible mechanism is inflammatory mechanism, by stimulation of cytokine production, induce thrombogenesis results in endothelial dysfunction and myocardial injury. [19,4]

VII. ANALYSIS OF RISK MORTALITY IN CARDIAC INJURY WITH BIOMARKERS

According to guidelines and protocols corona virus disease 2019 is divided into four stages depending on the severity of disease. All cases of corona virus disease are categorized into four categories. [6,7]

A. Mild Cases

Mild conditions appear with no pneumonia symptoms appear in this disease.

B. Ordinary Cases

In ordinary cases the patients have developed the symptom like fever, pneumonia and respiratory tract symptoms.

C. Severe cases

Respiratory distress and lesion progression is seen in the severe cases of disease.

D. Critical cases

The condition is characterized by the respiratory failure, other organ dysfunction, shift toward mechanical ventilation and proper monitoring is required. [6,7]

VIII. MECHANISM EXPLORATION

A. How Virus Infects?

Virus directly attack on cardio myocytes and replicates, causing degeneration of cardio myocytes which may disturb the function of cardiac tissue and arrhythmia. Virus specifically binds to the receptor cells and infects the target cells. [12,11,9] It may distort the function of cardiac cells and increased level of troponin T; CK iso-enzyme etc. has been observed which finally diagnose coronavirus fulminant myocarditis. [13]

Endo myocardial biopsy in an old age patient confirmed myocardial inflammation and viral particles in myocardium which showed either a viraemic phase or infect macrophage migration from lungs which linked to myocardial localization of SARS-COV-2. [10,11] Myocarditis is identified by viral load and it has been estimated that about 7 % of COVID-19 related deaths are due to myocarditis. [1,5]

B. Binding of functional receptors on cardio myocytes:

ACE2 (Angiotensin converting enzyme 2) is a mono-carboxylate which converts angiotensin II into (1-7) and (1-9) that is highly expressed in heart and lungs. ACE2 receptors associated with SARS invasion that may lead to myocarditis. [14,19,20] In binding form, it may present on membrane surface but during stress condition it may cleave from membrane surface by membrane-anchored metalloprotease (ADAM17) and enter into the circulatory system. Its elevated level may lead to abnormalities like hypertension, diabetes and cardiovascular injury. [19]

ACE2 cellular receptor regulates circulatory system but viral infection blocks target ACE2 or it may down regulate ACE2 on cell membrane thus leading to its abnormal secretion. ADAM17 highly present in lungs and heart may regulate ACE2 secretions and elevated level of ADAM17 promotes shedding and solubilizes ACE2 which may inhibit entry of virus in cells. [4] Therefore, further studies are required to determine the therapeutic effects of ACE2.

C. Damage to Immune System

Recently, it has been demonstrated, the patients infected with Corona virus have plasma containing many inflammatory cytokines like IL-7, TNF-alpha, IL-8 etc. and their level is high in patients that are seriously ill or deaths. Pathogenesis is characterized by high level of cytokine and cell division and produce over exuberant immunological host response. [19,20] Monocytes and macrophages activate inflammatory cells to release pro-inflammatory cytokines that causes congestive heart failure. It shows that cytokines involve in cardiac injury. [11]

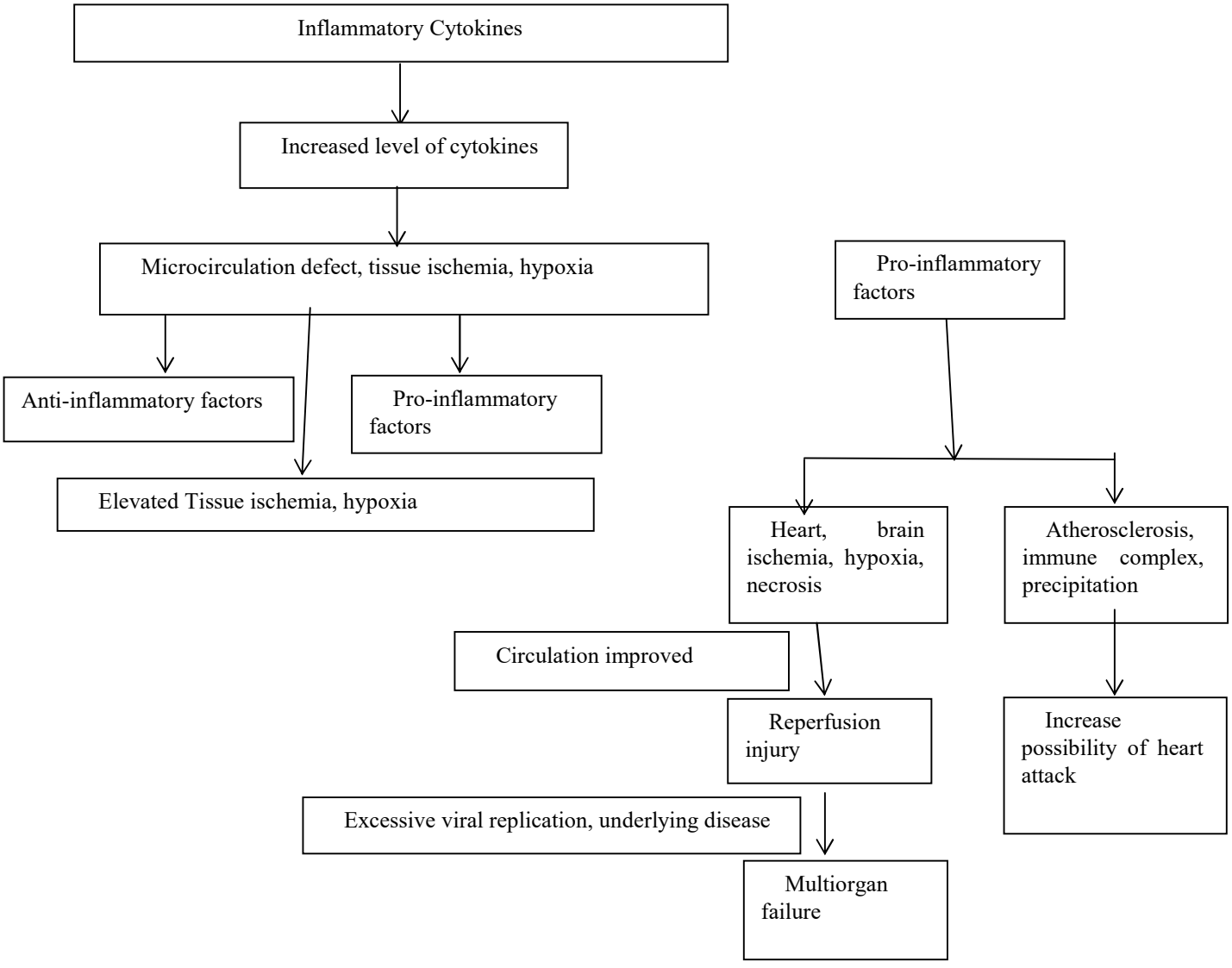
Inflammatory cytokines may act on WBCs and platelets to produce mediators that cause inflammation, which may increase blood C- reactive proteins (fibrinogen) by decreasing albumin and transferrin. Cytokines produce effects on capillaries causing ischemia and hypoxia in peripheral tissues. [16,19] When the level of cytokines decreases, peripheral and blood circulation becomes normal and release hypertension and tachycardia. As disease progress, viral load becomes more. If it returns to normal it may produce excessive immune response which may damage heart tissues and cause cardiac injury and increased level of cardiac markers. [18]

Patients suffering from COVID-19 have not well managed inflammatory cytokines and influx of calcium ions induced by a condition called hypoxia that may cause injury and apoptosis of cardio myocytes.Thus, poorly managed inflammatory cytokines may lead to atherosclerosis, it precipitates ischemic disease and may cause worsening in immunological response. [10,11,15]

IX. RESULTS

COVID – 19 Patients show severity in disease if they have pre-existing cardiovascular diseases

Systematic analysis shows that the patients suffering from pre-existing chronic cardiovascular diseases along with hypertension and diabetes develop more severe symptoms and these patients exhibit more severe mortality rate. [12] A study shows that patients suffering from chronic heart disease have increased level of troponin T (TnT), 49% Patients died in ICU due to severity in symptoms with viral infection. [1,11]



Although COVID-19 fist effects the lungs and cause interstitial pneumonitis, then leads to multi organ failure particularly cardiovascular system. SARS-COV-Positive patients usually show symptoms like chest pain as compared to respiratory problems. An examination of COVID-19 patient with sever heart damage shows an increased level of highly sensitive cardiac troponin I (hs-cTnI). [10,15,8] Moreover, the expression markers indicate serious cardiac damage like (CK-MB) and (NT-proBNP). The increased level of these parameters leads to higher mortality rate in COVID patients and this has been observed in non-survivors.[5]

A 63 years old patient suffering fromCOVID-19 exhibit fulminant myocarditis after five days of disease initiation and this condition of myocarditis occurs during higher viral load as myocarditis is related to higher viral load. Endo-myocardial biopsy revealed inflammation and viral particles in cardiac tissues. [6,7]

A.ACE2 Viral Receptor (A major contributor to cardiac involvement in COVID Patients)

ACE2 is important in protecting RAS (renin-angio-tensin system). It protects the function of tissues by converting angiotensin I and II into Ang (1-9) and Ang (1-7). It is located on external surface of cells, either in soluble or membrane binding form. [11] It has been estimated that the higher level of angiotensin II indicates heart failure and hypertension so ACE2 protects heart tissues by converting angiotensin II into less severe form. In COVID Patients ACE protein is reduced and the level of angiotensin II in plasma form is higher as compared to healthy individuals.[18]

So, increased angiotensin II level is related to viral infection and lung damage in COVID-19 Patients. Down-regulation of ACE2 will inhibit the cardio- protective role of Ang (1-7) which leads to increased level of inflammatory cytokines like TNF-alpha (involve in myocardial injury). [7,18] Many studies also showed that increased level of various inflammatory biomarkers like d-dimer, serum ferritin and IL-6 may increase the mortality rate in COVID patients as increased level of these biomarkers observed in non-survivors. [13]

B. Clinical Scenario of COVID -19 Patients regarding cardiac markers

The laboratory level of cardiac biomarkers like LDH, CK, CK-MB (muscle brain activity) and AST etc. have been observed in patients affected with Corona virus. AST CK and LDH are cardiac enzymes and their increased level may indicate cardiac injury. The level of these cardiac markers may be above to the normal level at the midpoint of hospitalization and this may spike before death. [13, 11, 16]

However, the clinical observations of Patients suffering from Corona have diseases likes cardiac MRI, Endo-myocardial biopsy is rarely feasible so elevated level of troponin associated with echocardiographic data is compatible with myocarditis. Change in ECG also contributes in indication of Myocarditis (ST elevation). Some laboratory cardiac markers return to normal and patients are improved but in case of severity, worsening in laboratory cardiac markers may lead to irreversible loss. [9] Persistent elevation of cardiac markers is a prognostic factor for infection. The incidence of cardiac injury is 13 folds higher in patients admitted in ICU as compared to non-ICU patients. [18]

C. Meta-analysis of COVID-19 Patients

Meta-analysis is very important as it includes sample size and global reach etc. Meta-analysis shows that COVID-19 patients having previous history of cardiovascular diseases are highly associated with mortality with elevated biomarkers. [16] Further analysis is done with serum biomarkers and it revealed that cTnI, AST and other serum biomarkers may have potential to elevate during viral infection and they may be predictors for risk. [12,16]

The exact reason by which elevated level of cardiac biomarkers that may increase mortality rate in COVID-19 patients is thrombosis, myocarditis and atherosclerotic plaque rupture. Therefore, biomarkers which show high inflammatory response may lead to microvascular damage and increase mortality rate.[18] Thus, elevated cardiac injury biomarkers may improve the mortality risk for COVID-19 patients, while identified earlier.

D. Trajectory pattern of Elevated cardiac biomarkers and inflammatory Factors in COVID Patients

A temporal relationship between cardiac biomarkers and inflammatory factors shows that there is cumulative proportion of patients with elevated cardiac biomarkers in association with inflammatory factors. Patients having cardiac injury have increased rate of cardiac biomarkers and inflammatory markers as compared to the patients that suffer from viral infection with no previous heart injury. Sharp increase of neutrophil percentage and CRP is observed in one set of disease. Significant increase of IL-6 observed when there is abnormality in cardiac markers. [20, 11,15] Patients having lower level of (NT-pro) BNP have much more favorable outcome as compared to the patients having elevated serum IL-6. The patients having increased level of inflammatory marker (CRP) have interactive effects with cardiac injury markers. Both of these are strongly associated with poor outcomes in COVID-19. [1,4,6, 17

REFERENCES

[1] Aldous, S. J. (2013). Cardiac biomarkers in acute myocardial infarction. *International Journal of Cardiology, 164*(3), 282–294. doi: 10.1016/j.ijcard.2012.01.081

[2] C Chen, C Chen, J T Yan, N Zhou, J P Zhao, D W Wang (2020). Analysis of myocardial injury in patients with COVID-19 and association

between concomitant cardiovascular diseases and severity of COVID-19, *pubmed.gov, 48*(7), 567-571. DOI: 10.3760/cma.j.cn112148-20200225-00123

[3] Du, Rong-Hui; Liu, Li-Min; Yin, Wen; Wang, Wen; Guan, Lu-Lu; Yuan, Ming-Li; Li, Yu-Lei; Hu, Yi; Li, Xu-Yan; Sun, Bing; Peng, Peng; Shi, Huan-Zhong (2020). Hospitalization and critical care of 109 decedents with COVID-19 pneumonia in Wuhan, China. *Annals of the American Thoracic Society, (),* doi:10.1513/AnnalsATS.202003-225OC

[4] Gao, Lei; Jiang, Dan; Wen, Xue-song; Cheng, Xiao-cheng; Sun, Min; He, Bin; You, Lin-na; Lei, Peng; Tan, Xiao-wei; Qin, Shu; Cai, Guo-qiang; Zhang, Dongying. (2020). Prognostic value of NT-pro BNP in patients with severe COVID-19. *Respiratory Research, 21*(1), 83, doi:10.1186/s12931-020-01352-w

[5] Grasselli, Giacomo; Zangrillo, Alberto; Zanella, Alberto; Antonelli, Massimo; Cabrini, Luca; Castelli, Antonio; Cereda, Danilo; Coluccello, Antonio; Foti, Giuseppe; Fumagalli, Roberto; Iotti, Giorgio; Latronico, Nicola; Lorini, Luca; Merler, Stefano; Natalini, Giuseppe; Piatti, Alessandra; Ranieri, Marco Vito; Scandroglio, Anna Mara; Storti, Enrico; Cecconi, Maurizio; Pesenti, Antonio (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA, (),* doi:10.1001/jama.2020.5394

[6] Haller, P. M., Beer, B. N., Tonkin, A. M., Blankenberg, S., & Neumann, J. T. (2020). Role of cardiac biomarkers in epidemiology and risk outcomes. *Clinical Chemistry.* doi:10.1093/clinchem/hvaa228

[7] Harris, J. E., Shah, P. J., Korimilli, V., & Win, H. (2019). Frequency of troponin elevations in patients with influenza infection during the 2017–2018 influenza season. *IJC Heart & Vasculature, 22,* 145–147. doi: 10.1016/j.ijcha.2018.12.013

[8] Hua, A., O'Gallagher, K., Sado, D., & Byrne, J. (2020). Life-threatening cardiac tamponade complicating myo-pericarditis in COVID-19. *European Heart Journal.* doi:10.1093/eurheartj/ehaa253

[9] Kuypers, J.; Martin, E. T.; Heugel, J.; Wright, N.; Morrow, R.; Englund, J. A. (2007). Clinical disease in children associated with newly described coronavirus subtypes. *PEDIATRICS, 119*(1), doi:10.1542/peds.2006-1406

[10] Li, Bo; Yang, Jing; Zhao, Faming; Zhi, Lili; Wang, Xiqian; Liu, Lin; Bi, Zhaohui; Zhao, Yunhe (2020). Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clinical Research in Cardiology, (),* doi:10.1007/s00392-020-01626-9

[11] Li, Lin; Zhou, Qi; Xu, Jiancheng (2020). Changes of laboratory cardiac markers and mechanisms of cardiac injury in coronavirus disease 2019. *BioMed Research International, 2020*(),1–7. doi:10.1155/2020/7413673

[12] Li, J.-W., Han, T.-W., Woodward, M., Anderson, C. S., Zhou, H., Chen, Y.-D., & Neal, B. (2020). The impact of 2019 novel coronavirus on heart injury: A systemic review and Meta-analysis. *Progress in Cardiovascular Diseases*. doi: 10.1016/j.pcad.2020.04.008

[13] Li, Xiaochen; Xu, Shuyun; Yu, Muqing; Wang, Ke; Tao, Yu; Zhou, Ying; Shi, Jing; Zhou, Min; Wu, Bo; Yang, Zhenyu; Zhang, Cong; Yue, Junqing; Zhang, Zhiguo; Renz, Harald; Liu, Xiansheng; Xie, Jungang; Xie, Min; Zhao, Jianping (2020). Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *Journal of Allergy and Clinical Immunology, (),* doi: 10.1016/j.jaci.2020.04.006

[14] Maisel, A. S., Bhalla, V., &Braunwald, E. (2006). Erratum: Cardiac biomarkers: a contemporary status report. *Nature Clinical Practice Cardiovascular Medicine, 3*(5), 288–288. doi:10.1038/ncpcardio0514

[15] McDonnell, B., Hearty, S., Leonard, P., &O'Kennedy, R. (2009). Cardiac biomarkers and the case for point-of-care testing. *Clinical Biochemistry, 42*(7-8), 549–561. doi10.1016/j.clinbiochem.2009.01.019

[16] Qin, J.-J., Cheng, X., Zhou, F., Lei, F., Akolkar, G., Cai, J., Li, H. (2020). Redefining cardiac biomarkers in predicting mortality of inpatients with COVID-19. *Hypertension*. doi:10.1161/hypertensionaha.120.15528

[17] Singh, V., Martinezclark, P., Pascual, M., Shaw, E.S., &O'Neill, W. W. (2010). Cardiac biomarkers – the old and the new: a review.*Coronary Artery Disease, 21*(4), 244–256. doi: 10.1097/mca.0b013e328338cd1f

[18] Suxin Wan, Yi Xiang, Wei Fang, Yu Zheng, Boqun Li, Yanjun Hu, Chunhui Lang, Daoqiu Huang, Qiuyan Sun, Yan Xiong, Xia Huang, JinglongLv, Yaling Luo, Li Shen, Haoran Yang, Gu Huang, Ruishan Yang. (2020). Clinical features and treatment of COVID-19 patients in northeast Chongqing, *Pubmed.gov, 92*(7), 797-806. doi: 10.1002/jmv.25783

[19] Toraih, E. A., Elshazli, R. M., Hussein, M. H., Elgaml, A., Amin, M. N., El-Mowafy, M, Fawzy, M. S. (2020). Association of cardiac biomarkers and comorbidities with increased mortality, severity, and cardiac injury in COVID-19 patients: A meta-regression and decision tree analysis. *Journal of Medical Virology.* doi:10.1002/jmv.26166

[20] Willeit, P., Welsh, P., Evans, J. D. W., Tschiderer, L., Boachie, C., Jukema, J. W., Sattar, N. (2017). High-sensitivity cardiac troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants. *Journal of the American College of Cardiology, 70*(5), 558–568. doi: 10.1016/j.jacc.2017.05.062